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Encl: Substitute Specification, Claim and Abstract
New Drawing Figures 3 and 4
Title page and page 437 - Webster's New Collegiate
Dictionary (1981)

136.0703

PEACH TREE 'V75024'

FIELD OF INVENTION

The present invention relates to a non-melting fleshed clingstone processing peach variety [*Prunus persica* (L.) Batsch] that is disease resistant, high quality, cold tolerant and productive.

BACKGROUND OF THE INVENTION

'V75024' originated at the Horticultural Research Institute of Ontario (now the Department of Plant Agriculture, University of Guelph) at Vineland Station, Ontario, Canada. It arose as a single selection from the seedling population of V75024 that was derived from a controlled cross of 'Suncling' (patent status unknown) and 'New Jersey Cling 81' (patent status unknown) made in 1975. The selection was made in 1981 and then was asexually propagated by budding on peach seedling rootstock in the research nursery at Vineland Station, Vineland, Ontario, Canada. Comparative records of performance of 'V75024' have been kept on its performance at this site since that date. The asexual propagation demonstrates that such reproduction of the characteristics of the tree are consistent and are established or transmitted through succeeding generations. The tree of this variety is self-pollinating and self fruitful.

SUMMARY OF THE INVENTION

The new and distinct non-melting fleshed clingstone processing peach, 'V75024', has distinguished itself as a processing peach. It is a high quality mid-to late season non-melting fleshed clingstone peach that produces high

quality mid-to late season fruit suitable for processing as a canned or frozen product. It ripens 2 days later than 'Babygold 5' (patent status unknown), the variety it most closely resembles. Further compared to 'Babygold 5', 'V75024' has improved disease resistance, improved tree growth habit, good vigour, good tree and bud tolerance to low temperatures during the winter, good fruit productivity and large sized fruit. It has superior resistance to bacterial spot disease, *Xanthomonas campestris* pv *pruni* (Smith) Dye. It has acceptable tolerance to blossom blight and brown rot of stone fruits, both caused by *Monolinia fructicola* (G. Went) Honey and to *Leucostoma* canker caused by *Leucostoma persoonii* Hohn. and *L. cincta* (FR.:Fr.). The moderately firm flesh of the fruit is of suitable texture, sugar and acid characteristics such that the canned and frozen products meet or exceed industry standards.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is a photographic illustration of the fruit of 'V75024' on the tree.

FIG. 2 is a photograph depicting the external and internal characteristics of the fruit of 'V75024'.

FIG. 3 is a photograph of a gel with a series of 5 SR markers for 'V75024' and reference varieties 'Babygold 5', 'Babygold 7' and 'Catherina'.

FIG. 4 is an overlay with the bands deleted by gel analysis.

DESCRIPTION OF VARIETY

'V75024' is a non-spur tree type of strong vigour similar to 'Catherina' (patent status unknown), 'Babygold 5' and

'Babygold 7' (patent status unknown). The trees are semi-horizontal being intermediate between 'Catherina' which is horizontal and 'Babygold 5' and 'Babygold 7' which are semi-erect. The leaf blade is of medium size, with a mean length (10 leaves measured) of 144.4 mm, a range of 130-160 mm, a standard deviation of 10.44 mm. The mean width (10 leaves measured) of 37.8 mm, a range of 34-47 mm, a standard deviation of 3.97 mm. These measurements are comparable to 'Catherina' but less than for either 'Babygold 5' or 'Babygold 7'. The leaf blade profile is flat comparable to 'Catherina' but dissimilar to the upfolded leaves of 'Babygold 5' and 'Babygold 7'. The leaf blade tip is recurved downwards, the same as comparative varieties. The angle at the base of the leaf blade is acute compared to the right angle of 'Catherina' and the obtuse angle of 'Babygold 5 and Babygold 7'. The angle at the tip of the leaf blade is acute compared to the right angle of 'Babygold 7'. The anthocyanin coloration of the leaf blade observed in mid-summer is absent. On a scale of 1 (absent) and 9 (present) the serration of the leaf blade of 'V75024' is a 4 compared to 2 in 'Catherina', 6 in 'Babygold 5' and 4 in 'Babygold 7'. The petiole length is rated as medium-long, longer than the comparative varieties. The nectaries are present on the petiole. The shape of nectaries on the petiole is kidney-shaped. The number of nectaries on the petiole is normally 2 as they are for 'Babygold 5' and 'Babygold 7' but there were more than 2 on the petioles of 'Catherina'.

'V75024' anthocyanin coloration on the flowering shoot observed in mid-summer was present and of medium intensity. The density of flower buds observed in dormant period are medium-dense while the density of 'Catherina' was dense and

'Babygold 5' and 'Babygold 7' were medium. The distribution of flower buds observed in dormant period are in groups of 2 or more. The time of the beginning of flowering is medium-late in 'V75024' and comparative varieties. The flower shape is campanulate in 'V75024', 'Babygold 5' and 'Babygold 7' and rosaceous in 'Catherina'. The calyx color is Burgandy in 'V75024', 'Babygold 5' and 'Babygold 7' and greenish yellow in 'Catherina'. The petal shape is elongated in 'V75024', 'Babygold 5' and 'Babygold 7' and rounded in 'Catherina'. The petal size is medium in 'V75024', 'Babygold 5' and 'Babygold 7' and large in 'Catherina'. The petal color is medium pink in 'V75024' and the comparative varieties. Petal numbers are 5 and petals are lightly striped in 'V75024' and comparative varieties. All had one pistil per flower. The position of the stigma compared to the anthers is above in 'V75024', at the same level in 'Catherina' and slightly above in 'Babygold 5' and 'Babygold 7'. Anthers in flowers in 'V75024' and all comparative varieties produced a moderate amount of pollen.

The time of fruit maturity for 'V75024' is late, medium in Catherina, medium-late in 'Babygold 5' and very late in 'Babygold 7'. Fruit size is rated large for 'V75024', 'Catherina' and 'Babygold 7' and very large in 'Babygold 5'. Fruit shape is rounded in 'V75024', 'Catherina', 'Babygold 5' and ovate in 'Babygold 7'. The shape of the fruit tip is dimpled in all but 'Babygold 7' which is tipped. Fruit of all were symmetrical along the suture. 'V75024' had a more prominent suture than comparative varieties. The depth of the petiole cavity was medium-shallow for 'V75024', medium for 'Catherina' and 'Babygold 7' and shallow for 'Babygold 5'. The width of the petiole cavity is medium-narrow for 'V75024' and 'Catherina' and narrow for 'Babygold 5' and 'Babygold 7'.

The fruit skin ground color is cream-yellow in 'V75024', orange-yellow in 'Catherina', and greenish-yellow in 'Babygold 5' and 'Babygold 7'. The anthocyanin color of the skin of the fruit is dark red in 'V75024', orange red in 'Catherina' and pink-red in 'Babygold 5' and 'Babygold 7'. The pubescence on the skin of the fruits of 'V75024' is medium-dense and medium-sparse in the comparative varieties. The flesh of all is non-melting with firmness of 'V75024' being firm, 'Catherina' medium-firm and 'Babygold 5' and 'Babygold 7' medium. The ground color of the flesh is yellow to orange-yellow. Fruit anthocyanin coloration at the pit is present in 'V75024', 'Babygold 5' and 'Babygold 7' but absent in 'Catherina'.

The size of the stone of 'V75024' is medium and medium-small for 'Catherina' and medium-large for 'Babygold 5' and 'Babygold 7'. The stone shape is ovoid for 'V75024', 'Babygold 5' and 'Babygold 7', but globular for 'Catherina'. The stone color is red for 'V75024', 'Babygold 5' and 'Babygold 7', but tan for 'Catherina'. The percentage of split or shattering of the pits of 'V75024' and all of the comparative varieties is absent or very low. The flesh adherence to the pit is present and all are considered clingstone varieties.

'V75024', 'Babygold 5' and 'Babygold 7' 'Catherina' all are moderately resistant to *Leucostoma* canker disease. 'V75024' and 'Catherina' are resistant to bacterial spot disease caused by *Xanthomonas campestris* pv *pruni* (Smith) Dye but 'Babygold 5' is susceptible and 'Babygold 7' is moderately susceptible.

Table 1 provides a summary of the description of the claimed variety. A rating system of 1-9 provides a scale for describing most characteristics in this table.

Characteristics have been rated by selecting a value that best corresponds to the state indicated. Characteristics were rated with intermediate values where the characteristic grades gradually from one extreme to another. For example, where the states for a characteristic are described as: small (3), medium (5), large (7); other values of 1, 2, 4, 6, 8 or 9 may be selected.

Each characteristic in Table 1 has been arranged in a format allowing the claimed variety (CV) and three reference varieties (R1 to R3) to be described and compared.

Each characteristic is described for two years of observation. A rating (1-9) is given for each year, or a measurement including the standard deviation described for both years. An overall summary is presented as an average of both years.

CV: Claimed variety

R1-R3: Reference varieties

The age of the observed trees was about eight years.

(i)

REFERENCE VARIETIES

R1	R2	R3	R4
Catherina	Babygold 5	Babygold 7	

- (ii) Testing location: Vic farm, Department of Plant Agriculture -
Vineland Station, University of Guelph, 4890, Victoria
Avenue N. PO box 7000, Vineland Station, ON L0R2E0
- (iii) Protocols used for the tests and trials (plot size, number of
plants, number of replications, plant spacing, etc.)

Four trees of each -CV and references- were planted at a spacing of 12 feet within the rows and 20 feet between the rows.

Substitute Specification, Claim and Abstract

1.0 Denomination: proposed variety name: 'V75024

2.0 TREE/SHRUB CHARACTERISTICS

2.1 Tree: type

(*)

		CV	R1	R2	R3	
normal	1	1	1	1	1	
Spur	2					

2.2 Tree/shrub: vigor (in terms of height and width of crown)

		CV	R1	R2	R3	
very weak	1	5	5	4	5	
Weak	3					
Medium	5					
Strong	7					
very strong	9					

2.3 Tree/shrub: habit

(*)

		CV	R1	R2	R3	
Erect	1	4	5	3	3	
semi-erect	3					
horizontal	5					
drooping	7					
strongly drooping	9					

2.4 Tree/shrub: bark color (observed in mid-summer)

		CV	R1	R2	R3	
golden yellow	1	4 [188C]	4 [199A on 166B]	4 [197B]	4 [198A]	
Orange	2					
Green	3					
other (describe): <u>Grey*</u> [RHS color chart reference]	4					

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2.5 Tree/shrub: winter bark color (observed when dormant)

		CV	R1	R2	R3	
Green	1	4 [197A]	4 [199B on 166B]	4 [198B]	4 [198A]	
yellow-green	2					
Red	3					
other (describe): Grey* [RHS color chart reference]	4					

3.0 LEAF BLADE CHARACTERISTICS

3.1 Leaf blade: size

(*)

		CV	R1	R2	R3	
very small	1	5	5	7	7	
Small	3					
Medium	5					
Large	7					
very large	9					

3.2 Leaf blade: length (mm)

(*)

	CV	R1	R2	R3	
Mean	144.4	143.7	151.5	156.8	
Range	130-160	131-150	127-165	147-175	
standard deviation	10.44	5.44	10.35	9.10	
no. measured	10	10	10	10	

3.3 Leaf blade: width (mm)

(*)

	CV	R1	R2	R3	
Mean	37.8	36.5	46.0	43.0	
Range	34-47	32-40	40-48	38-47	
standard deviation	3.97	2.45	2.87	2.81	
no. measured	10	10	10	10	

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3.4 Leaf blade: profile

		CV	R1	R2	R3	
up-folded	1	2	2	1	1	
Flat	2					

3.5 Leaf blade: tip

		CV	R1	R2	R3	
in the plane of the leaf	1	2	2	2	2	
recurved downwards	2					

3.6 Leaf blade: angle at base

		CV	R1	R2	R3	
Acute	1	1	2	3	3	
right angle or nearly right angle	2					
Obtuse	3					

3.7 Leaf blade: angle at tip

		CV	R1	R2	R3	
Acute	3	3	3	3	2	
right angle or nearly right angle	5					
Obtuse	7					

3.8 Leaf blade: anthocyanin coloration (observed in mid-summer) (*)

		CV	R1	R2	R3	
Absent	1	1	1	1	1	
present	9					

3.9 Leaf blade: serration (*)

		CV	R1	R2	R3	
absent	1	4	2	6	4	
present	9					

3.10 Petiole: length

		CV	R1	R2	R3	
very short	1	6	4	5	5	
short	3					
medium	5					
long	7					
very long	9					

3.11 Petiole: nectaries

		CV	R1	R2	R3	
absent	1	4	6	5	5	
present	9					

3.12 Petiole: shape of nectaries

		CV	R1	R2	R3	
round	1	2	2	2	2	
kidney-shaped	2					

3.13 Petiole: number of nectarines

		CV	R1	R2	R3	
normally two	1	1	2	1	1	
normally more than two	2					

4.0 FLOWERING SHOOT CHARACTERISTICS**4.1 Flowering shoot: anthocyanin coloration (observed in mid-summer)**

		CV	R1	R2	R3	
absent	1	5	5	5	5	
present	9					

4.2 Flowering shoot: intensity of anthocyanin coloration (observed in mid-summer)

(*)

		CV	R1	R2	R3	
very weak	1	5	5	4	6	
weak	3					
medium	5					
strong	7					
very strong	9					

4.3 Flowering shoot: density of flower buds (observed in dormant period)

(*)

		CV	R1	R2	R3	
very sparse	1	6	7	5	5	
sparse	3					
medium	5					
dense	7					
very dense	9					

4.4 Flowering shoot: distribution of flower buds (observed in dormant period)

		CV	R1	R2	R3	
generally isolated	1	2	2	2	2	
generally in groups of two or more	2					

5.0 FLOWER CHARACTERISTICS**5.1 Time of beginning of flowering**

(*)

		CV	R1	R2	R3	
very early	1	6	6	6	6	
early	3					
medium	5					
late	7					
very late	9					

5.2 Flower: shape

(*)

		CV	R1	R2	R3	
rosaceous	1	2	1	2	2	
campanulate	2					

5.3 Calyx: color (observed when flower was open before petals fell)

(*)

		CV	R1	R2	R3	
greenish yellow	1	3 [60-D]	1 [147-B]	3 [60-C]	3 [60-D]	
orange	2					
other (describe) <u>Burgandy-red*</u> [RHS <u>color chart number</u> <u>included]</u>	3					

5.4 Petal: shape

(*)

		CV	R1	R2	R3	
rounded	1	2	1	2	2	
elongated	2					

5.5 Petal: size

(*)

		CV	R1	R2	R3	
very small	1	3	8	4	3	
small	3					
medium	5					
large	7					
very large	9					

5.6 Petal: color (at full flowering)

(*)

		CV	R1	R2	R3	
White	1	4 [56-C]	3 [49-D]	4 [55-C]	4 [56-D]	
very light pink	2					
light pink	3					
medium pink	4					
dark pink	5					
violet pink	6					
Red	7					
other (describe) [RHS color chart number included]	8					

5.7 Petal: striping

		CV	R1	R2	R3	
absent	1	4	2	4	4	
present	9					

5.8 Petal: number

(*)

		CV	R1	R2	R3	
five	1	1	1	1	1	
more than five	2					

5.9 Stamens: length compared to petals (observe at beginning of anther dehiscence)

		CV	R1	R2	R3	
shorter	3	3	3	3	5	3
equal	5					
longer	7					

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5.10 Pistils: number

		CV	R1	R2	R3	
always one	1	1	1	1	1	
sometimes more than one	2					

5.11 Stigma: position compared to anthers

(*)		CV	R1	R2	R3	
below	3	7	5	6	6	
same level	5					
above	7					

5.12 Anthers: pollen

(*)		CV	R1	R2	R3	
absent	1	6	5	6	6	
present	9					

5.13 Ovary: pubescence

(*)		CV	R1	R2	R3	
absent (nectarine)	1	9	9	9	9	
present (peach)	9					

5.14 Duration of flowering

(*)		CV	R1	R2	R3	
very short	1	3	3	3	3	
short	3					
medium	5					
long	7					
very long	9					

6.0 FRUIT CHARACTERISTICS**6.1 Time of maturity**

(*)

		CV	R1	R2	R3	
very early	1	7	5	6	8	
early	3					
medium	5					
late	7					
very late	9					

6.2 Type of picking season

		CV	R1	R2	R3	
short	1	1	1	1	1	
long	2					

6.3 Tendency to natural falling of fruits

		CV	R1	R2	R3	
absent or very weak	1	4	3	5	7	
weak	3					
medium	5					
strong	7					
very strong	9					

6.4 Fruit: size

(*)

		CV	R1	R2	R3	
Very small	1	7	7	8	7	
small	3					
medium	5					
large	7					
Very large	9					

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6.5 Fruit: shape (in profile view)

(*)

		CV	R1	R2	R3	
Very flat	1	3	3	3	4	
slightly flat	2					
rounded	3					
ovate	4					
oblong	5					
other (describe) _____ _____	6					

6.6 Fruit: shape of tip

(*)

		CV	R1	R2	R3	
dimpled	1	1	1	1	3	
smooth	2					
tipped	3					

6.7 Fruit: symmetry along the suture

		CV	R1	R2	R3	
asymmetric	1	2	2	2	2	
symmetric	2					

6.8 Fruit: prominence of suture

		CV	R1	R2	R3	
low	3	2	1	1	1	
medium	5					
high	7					

6.9 Fruit: depth of petiole cavity

		CV	R1	R2	R3	
shallow	3	4	5	3	5	
medium	5					
deep	7					

6.10 Fruit: width of petiole cavity
(*)

		CV	R1	R2	R3	
very narrow	1	4	4	3	3	
narrow	3					
medium	5					
broad	7					
very broad	9					

6.11 Fruit: ground color of skin
(*)

		CV	R1	R2	R3	
Green	1	6 [1-D]	8 [16-C]	5 [13-D]	2 [4-B]	
cream-green	2					
Cream	3					
pink-white	4					
greenish-yellow	5					
cream-yellow	6					
yellow	7					
orange-yellow	8					
other (describe): [RHS color chart number included]	9					

6.12 Fruit: anthocyanin coloration of skin

(*)		CV	R1	R2	R3	
dark red	1	1 [45-D]	4 [33-B]	3 [48-C]	3 [48-C]	
bright red	2					
pink red	3					
orange red	4					
Pink	5					
other (describe): [RHS color chart number included]	6					

6.13 Fruit: proportion of fruit surface with anthocyanin coloration on the skin

		CV	R1	R2	R3	
none (0%)	1	7	2	4	4	
light ($\pm 25\%$)	3					
medium ($\pm 50\%$)	5					
Heavy ($\pm 75\%$)	7					
very heavy ($> 75\%$)	9					

6.14 Fruit: characteristics of anthocyanin coloration

		CV	R1	R2	R3	
uniform	1	2	2	2	2	
punctuated	2					
striated	3					
marbled	4					

6.15 Fruit: pubescence

(*)		CV	R1	R2	R3	
absent (nectarine)	1	9	9	9	9	
present (peach)	9					

6.16 Fruit: density of pubescence**(*)**

		CV	R1	R2	R3	
very sparse	1	6	4	4	4	
sparse	3					
medium	5					
dense	7					
very dense	9					

6.17 Fruit: thickness of skin

		CV	R1	R2	R3	
very thin	1	5	4	4	5	
thin	3					
medium	5					
thick	7					
very thick	9					

6.18 Fruit: adherence of skin to flesh

		CV	R1	R2	R3	
absent or very weak	1	8	8	8	9	
weak	3					
medium	5					
strong	7					
very strong	9					

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6.19 Fruit: firmness of flesh

(*)		CV	R1	R2	R3	
very soft	1	7	6	5	5	
soft	3					
medium	5					
firm	7					
very firm	9					

6.20 Fruit: ground color of flesh

(*)		CV	R1	R2	R3	
White	1	2 [18-A]	2 [21-B]	2 [19-B]	2 [7-C]	
yellow to orange yellow	2					
Red	3					
other (describe) [RHS color chart number included]	4					

6.21 Fruit: anthocyanin coloration directly under skin

(*)		CV	R1	R2	R3	
Absent	1	1	1	1	1	
Present	9					

6.22 Fruit: anthocyanin coloration of the flesh

(*)		CV	R1	R2	R3	
Absent	1	1	1	1	1	
Present	9					

6.23 Fruit: anthocyanin coloration around the stone

(*)		CV	R1	R2	R3	
Absent	1	9	1	9	9	
present	9					

6.24 Fruit: stringiness of the flesh

		CV	R1	R2	R3	
not stringy	1	1	1	1	1	
more or less stringy	2					

6.25 Fruit: texture of the flesh

		CV	R1	R2	R3	
melting	1	2	2	2	2	
non-melting	2					

6.26 Fruit: acidity of flesh

		CV	R1	R2	R3	
very sweet	3	5	5	6	5	
intermediate	5					
very acid	7					

6.27 Fruit: juiciness of flesh

		CV	R1	R2	R3	
Dry	3	5	5	5	5	
intermediate	5					
Juicy	7					

7.0 STONE CHARACTERISTICS

7.1 Stone: size compared to fruit
(*)

		CV	R1	R2	R3	
small	3	5	4	6	6	
medium	5					
large	7					

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7.2 Stone: shape (in same plane as suture)

(*) (+)

		CV	R1	R2	R3	
flattened	1	3	2	3	3	
globular	2					
ovoid	3					
elongated	4					
other (describe): _____	5					

7.3 Stone: color (observed once dry)

(*) Rootstock varieties only

		CV	R1	R2	R3	
Tan	1	2	1	2	2	
Red	2					

7.4 Stone: percentage of split or shattered stones

		CV	R1	R2	R3	
absent or very low	1	1	1	1	1	
low	3					
medium	5					
high	7					
very high	9					

7.5 Stone: adherence to flesh

(*)

		CV	R1	R2	R3	
absent	1	9	9	9	9	
present	9					

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7.6 Stone: degree of adherence to flesh

		CV	R1	R2	R3	
slight	3	7	7	7	7	
medium	5					
strong	7					

8.0 REACTION TO DISEASES

- 0 - not tested
 1 - resistant
 3 - moderately resistant
 5 - moderately susceptible
 7 - susceptible
 9 - very susceptible

		CV	R1	R2	R3	
8.1	Crown gall - (<i>Agrobacterium tumefaciens</i>)	0	0	0	0	
8.2	Canker (<i>Cytospora</i> spp.)	4	3	4	4	
8.3	Brown rot (<i>Monilinia fructicola</i>)	0	0	0	0	
8.4	Powdery mildew (<i>Sphaerotheca pannosa</i> var. <i>persicae</i>)	0	0	0	0	
8.5	Peach leaf curl (<i>Taphrina deformans</i>)	0	0	0	0	
8.6	Bacterial spot (<i>Xanthomonas campestris</i> pv. <i>pruni</i>)	2	2	7	5	
8.7	Peach yellows virus	0	0	0	0	
8.8	X-disease	0	0	0	0	
8.9	Other (please specify): _____ _____ _____					

9.0 Reaction to winter cold during the dormant period

- 9 - hardy - injury confined to previous season's growth
 7 - medium hardy - injury confined to previous two season's growth
 5 - medium tender - two major scaffold branches dead
 3 - tender - four major scaffold branches dead
 1 - very tender - tree dead or dying

	CV	R1	R2	R3	R4
winter cold reaction	8	8	8	7	

- 10.0 Other characteristics which are important for differentiating the candidate variety from the reference varieties.

Distinguishes well at several microsatellite (SSR) loci against the reference varieties. See section below on DNA fingerprinting of peach varieties using microsatellite markers.

- 11.0 The characteristics which are most useful in distinguishing the claimed variety from the reference varieties. Refer to the characteristics using the objective description key numbers.

The claimed variety has large fruits as in the reference varieties. Fruits are rounded and dimpled. Compared to Babygold5 [R2], it has been disease resistance to bacterial spot [*Xanthomonas campestris* pv. *Pruni*] and moderately tolerant to blossom blight and brown rot. [*Monolinia fruticola*] Further it matures at least 2 days later than Babygold 5 [R2] at the test site.

**DNA fingerprinting of peach (*Prunus persica*) varieties
using microsatellite markers**

Materials and methods

DNA extraction

Young leaf tissue samples of V75024, Babygold 5, Babygold 7, and Catherina were collected in May and September 2003 from nurseries located near Vineland, Ontario. The tissue was immediately frozen in liquid nitrogen, transported on dry ice and frozen at -80°C. DNA was extracted according to Serrano et al., 2002 with some modifications. Approximately 50mg of leaf tissue were ground in a Fast Prep machine (QBioGene) with 200uL extraction buffer (100mM Tris-HCl, 1.4M NaCl, 20mM EDTA, 2% CTAB, 1% PVP, 0.2% β -mercaptoethanol, 0.1% NaHSO₃) and incubated at 65°C for 30 minutes. An equal volume of chloroform:isoamyl alcohol (24:1) was added, mixed by inversion and centrifuged at 6,000 x g for 15 minutes. The supernatant was transferred to a new tube, mixed with 10ug RNase A and incubated at 37°C for 30 minutes. An aliquot of

130uL ice-cold isopropanol was added, mixed by inversion and centrifuged at 13,000 x g for 5 minutes to recover the nucleic acid precipitate. The supernatant was removed and the pellet was washed with 400uL of 10mM ammonium acetate in 76% ethanol for 10 minutes and dried at room temperature. The pellet was re-suspended overnight at 4°C in 200uL of sterile ddH₂O (pH 8.0) and quantified using a Spectramax Plus spectrophotometer (Molecular Devices). The DNA was then stored at -20°C.

PCR amplification

Microsatellite primer pairs previously developed in peach (Table 1) were synthesized by Sigma Genosys (Oakville, ON). PCR was performed in a Robocycler 96 (Stratagene) and consisted of one incubation of 5 minutes at 94°C followed by 35 cycles of 45 seconds at 94°C, 45 seconds at the appropriate annealing temperature (Table 1) and 1 minute at 72°C. The final cycle was followed by a 5 minute incubation at 72°C. Amplification reactions were carried out in 25uL volumes containing 10mM Tris-HCl pH 8.3, 50mM KCl, 1.5mM MgCl₂, 100uM of each dNTP, 10pmol of each primer, 1U Taq polymerase (Sigma) and approximately 200ng peach genomic DNA. Following amplification, 10uL of the PCR reaction was separated on a 1% agarose gel containing ethidium bromide to verify PCR amplification. Samples were then stored at -20°C prior to polyacrylamide gel electrophoresis.

Table 2. List of microsatellite markers (SSRs) used to identify peach varieties

SSR	Sequence (5'-3')	Annealing temperature (°C)	Reference
CPPCT030	TGAATATTGTTCTCAATTC CTCTAGGCAAGAGATGAGA	50	Aranzana et al., (2002)
Pchcms2	AGGGTCGTCTCTTTGAC CTTCGTTTCAAGGCCTG	50	Sosinski et al., (2000)
Pchcms5	CGCCCATGACAACTTA GTCAAGAGGTACACCAG	50	Sosinski et al., (2000)
UDP96-013	ATTCTTCACTACACGTGCACG CCCCAGACATACTGTGGCTT	57	Cipriani et al., (1999), Testolin et al., (2000)
UDP98-407	AGCGGCAGGCTAAATATCAA AATCGCCGATCAAAGCAAC	52	Cipriani et al., (1999), Testolin et al., (2000)
BPPCT025	TCCTGCGTAGAAGAAGGTAGC CGACATAAAGTCCAAATGGC	57	Dirlewanger et al., (2002)
Pchgms1	GGGTAAATATGCCCATTTGTGCAATC GGATCATTGAACTACGTCAATCCTC	52	Sosinski et al., (2000)

Visualization of microsatellites

The PCR products were denatured by addition of an equal volume of sample buffer (95% de-ionized formamide, 10mM NaOH, 0.05% bromophenol blue, 0.05% xylene cyanol). Samples were heated at 94°C for 6 minutes and immediately placed on ice. Two microlitres of each denatured preparation were loaded onto a 38x50cm 6% polyacrylamide sequencing gel containing 7M urea in 1X TBE buffer (90mM Tris, 90mM boric acid, 1mM EDTA). Gels were run on a Sequi-Gen GT Nucleic Acid Electrophoresis Cell

(Bio-Rad) for 4-5 hours at 85W. Following electrophoresis, gels were silver-stained using the SilverXpress Silver Staining Kit (Invitrogen), scanned on a HP scanjet 5470c scanner and dried on a Slab Gel Dryer SGD2000 (Savant). Fragment sizes were determined with 10-bp and 25-bp ladder DNA sizing markers (Invitrogen) using The Discovery Series, Quantity One software program (Bio-Rad).

Results

The microsatellite primer pairs revealed genetic differences between and among the four peach varieties (Figures 1 and 2). Table 3 summarizes the fragment sizes detected for each of the primer pairs used based on repeated samples. There are a number of fragments that can be used to distinguish each of the four varieties using pairwise or any combination of comparisons. For example, fragments 262/264 from SSR BPPCT025 which are present in V75024, Catherina and Babygold 7 are not detected in Babygold 5. The characteristic fragment sizes that can be used to distinguish V75024 from all three reference varieties are presented in bold type in Table 2. These characteristic fragments are fragment 255 using SSR Pchcms2 and fragments 143, 142, 141, 140, 139, 138, 137, and 136 using SSR UDP96-013. These fragments are produced from DNA extracts of V75024 but are absent in Babygold 5, Babygold 7, and Catherina.

Table 3. Fragment sizes (bp) detected for four peach varieties using seven microsatellite primer pairs. Fragment sizes listed in **bold** are unique fragment sizes detected in V75024 that are not detected in the other three varieties.

SSR	Peach Variety			
	V75024	Babygold 5	Babygold 7	Catherina
CPPCT030	200,198, 196,195, 190,189, 187,185	190,188, 187, 184	191,190, 188, 184	200,198, 197,195, 190,189, 187,185
Pchcms2	255,245	245	246	246
Pchcms5	183,181, 174,171, 169,166, 164	183,181, 174,171, 170,166, 164	183,181, 174,171, 169,166, 164	183,181, 174,171, 169,166, 164
UDP96-013	143,142, 141,140, 139,138, 137,136	197,196, 194, 192	197,195, 194, 191	197,196, 194, 192
UDP98-407	205,204, 202,200, 190,182, 181	204,202, 200,190, 182,181	207,205, 203, 190	205,203, 202,200, 190
BPPCT025	304,265, 263,204, 202,200, 198,196, 194, 193	304,281, 279,204, 202,200, 198,197,194	312,263, 262,203, 201,199, 197,195,194	305,264, 262,204, 202,200, 198,196, 194, 192
Pchgms1	191,190	192,190	192,190	192,190

Figure 3. Silver stained 6% polyacrylamide sequencing gel of four peach cultivars (1=Babygold 5, 2=V75024, 3=Catherina, 4=Babygold 7) using seven microsatellites (A=pchgms1, B=BPPCT025, C=UDP98-407, D=UDP96-013, E=pchcms5, F=pchcms2, G=CPPCT030).

Figure 4. Silver stained 6% polyacrylamide sequencing gel of four peach cultivars (1=Babygold 5, 2=V75024, 3=Catherina, 4=Babygold 7) using seven microsatellites (A=pchgms1, B=BPPCT025, C=UDP98-407, D=UDP96-013, E=pchcms5, F=pchcms2, G=CPPCT030) showing banding patterns identified using Quantity One software.

Methods of fingerprinting peach varieties are well known in the art. For example, see Aranzana M.J., Garcia-Mas, J., Carbo, J. and P. Arus. 2002. Development and variability analysis of microsatellite markers in peach. Plant Breeding. 121:87-92; Cipriani G., Lot G., Huang W-G., Marrazzo M.T., Peterlunger E. and R. Testolin.1999. AC/GT and AG/CT microsatellite repeats in peach [*Prunus persica* (L) Batsch]: isolation, characterization and cross-species amplification in *Prunus*. Theor. Appl. Genet. 99:65-72; and Serrano B., Gomez-Aparisi J and J.I. Hormaza. 2002. Molecular fingerprinting of *Prunus* rootstocks using SSRs. J. Hort. Sci. Biotech. 77(3):368-372.